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STANDARDIZATION OF DISEASE SCREENING PROTOCOL FOR SHEATH ROT DISEASE IN RICE

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ABSTRACT

Seed inoculation of rice varieties of BPT-5204, MAS-26 and MAS 946-1 by soaking of seeds in conidial suspension (10^5 condial/ml) of Sarocladium oryzae overnight was carried out in two factorial complete randomized design (CRD). Significant differences were observed among inoculation techniques (inoculated vs control) and among varieties as indicated by reduced plant in seed inoculated plants as compared to that of control. The detached tiller assay experiment was carried out three factorial CRD showed significant differences among inoculation techniques and varieties. Enlarged lesion (3.25 cm) was observed in susceptible variety BPT-5204 within 14 days after inoculation as compared to resistant variety HP-14 (1.4 cm). Foliar inoculation and cotton swabbing with conidial suspension (10^5 conidia/ml) during peak vegetative stage to booting stage in BPT-5204 showed enlarged lesion whereas resistant breeding line HP-14 took no infection and found highly resistant to sheath rot disease. In attached tiller assay and standard grain inoculation, lesion development was observed within 15 days after inoculation. Development of sheath rot lesion was observed within 72 hours of inoculation with mycelial mat in detached leaf sheath assay and detached leaf assay. Seed inoculation, detached tiller assay and foliar inoculation with conidial suspension (10^5 conidia/ml) were recommended for large scale screening of germplasm, breeding lines and phenotyping of mapping population.

KEYWORDS: Standardization of Disease Screening, BPT-5204, MAS-26 and MAS 946-1 by Soaking

INTRODUCTION

Sheath rot disease of rice caused by *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw] has emerged as one of the major disease affecting crop in almost all rice-growing ecosystems of the world. Sheath rot is a serious menace to rice cultivation, causing yield losses ranging from 3 to 85 per cent depending upon disease severity. *Sarocladium oryzae* is primarily seed borne and survives on plant debris and weeds, disseminates conidia through wind and sucking pests. Pathogen produces phytotoxins *viz.*, cerulenin and helvoic acid which are responsible for production of characteristic greyish-brown lesion on flag leaf sheath and discolouration of grains (Amin *et al*, 1974, Chakravarthy and Biswas, 1978). Dwarf and semi dwarf varieties associated with partially exerted panicle are more susceptible as compared to tall varieties with full panicle exerted varieties. (Hittalmani *et al.*, 2000 and Srinivasachary *et al*, 2002). Therefore, sheath rot disease can effectively managed through crop improvement strategies *viz.*, discovery of resistance sources from varieties, germplasm, land races, wild genetic resources and further deploying at genotypic and cropping system could be effective in mitigation of disease. It is advantageous and important to employ appropriate disease screening methods to identify resistant sources in germplasm, land races, breeding lines and wild genetic resources. In the

present study, various method of disease screening protocols were standardized for sheath rot disease screening.

MATERIALS AND METHODS

Material

Sheath rot causing fungus *S. oryzae* was isolated from diseased sheath sampled from experimental blocks of Dept of Genetics and Plant Breeding, University of Agricultural Sciences, Bangalore (Saro-24) during *kharif* season, 2013. Disease sheath was surface sterilized using 0.05 per cent mercuric chloride followed by three times washing with sterile millipore water. Sterilized diseased sheath pieces were incubated for 4-5 days on sterile petriplates with moist whatman filter paper at room temperature. Germinating colonies were transferred to potato dextrose agar medium and *S. oryzae* was identified based on morphology of conidiophores, phialades and conidia (Ou, 1985). The fungus was characterized for internal transcriber region using ITS-4 and ITS-5 markers (gene bank ID: KT291728; Schoch *et al.*, 2012). Isolate Saro-24 was mass multiplied on potato dextrose agar and potato dextrose broth and used for standardization of disease screening protocols.

METHODOLOGY

Seed Inoculation Techniques

The experiment was conducted with two factor *viz.*, seed treatment with two levels (inoculation *vs* control) and varieties with three levels (BPT-5204, MAS-26 and MAS-946-1) was carried out in two factorial complete randomized design (CRD) with five replication in polyhouse, Dept of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK, Bangalore during *kharif* season, 2013. Seeds of BPT-5204, MAS-26 and MAS-946-1 were soaked in conidial suspension overnight (GKVK isolate, Saro-24, gene bank ID: KT291728) and seeds were in pots. Observation of plant height was recorded in 15^{th} days after sowing.

Detached Tiller Assay

An experiment was conducted in three factorial complete block design with two levels each *viz.*, inoculated *vs* control, flag leaf sheath injured *vs* not injured and varieties (BPT-5204 and HP-14). The experiment was carried out under laboratory condition (25 ± 2^0C) at Marker Assisted Laboratory (MAS Lab), Dept. of Genetics and Plant Breeding, University of Agricultural Sciences, Bangalore Seven days old culture of *S. oryzae* (GKVK isolate, Saro-24, gene bank ID: KT291728) cultured on potato dextrose agar (HiMedia ID: M-096) used in the experiments. Mycelial mat on potato dextrose agar medium was cut into approximately equal size using sterile blade and used for inoculation purpose (Plate 1). Tillers at booting stage were detached from main plant and flag leaf sheath was inoculated with mycelial disc, covered with sterile moistened absorbent cotton and wrapped with paraffin film. Inoculated tillers were placed in millipore water and water level was maintained covering first nodal region under laboratory condition (25 ± 2^0C). Observations recorded on 14^{th} days after inoculation for lesion length (cm) and length of panicle discolouration (cm). The pathogen was re-isolated from infected sheath to confirm the pathogenicity

Foliar Inoculation and Cotton Swabbing with Conidial Suspension

S. oryzae (GKVK Isolate, Saro-24, gene bank ID: KT291728) was mass multiplied on potato dextrose agar medium, potato dextrose broth and rice kernel methods were used in the study. Seedlings of BPT-5204 and HP-14 sown on

pots in polyhouse at 'K' block, University of Agricultural Sciences, GKVK, Bangalore

Plate 1: Steps Involved in Detached Tillers Assay
Method of Screening for Sheath Rot Disease in Rice
A: Inoculation with Mycelia B. Wrapping with Paraffin Film
C: Incubation in Room Temperature D & E: Nodal Rooting Induced
During Incubation F: Infected Sheath

During late *kharif* season, 2013. Conidial suspension (10^5 conidia/ml) was sprayed on 40^{th}, 50^{th}, 60^{th} and 70^{th} days after sowing during grand vegetative and booting stage and flag leaf sheath was cotton swabbed with conidial suspension (10^5 conidia/ml)during booting stage. Sheath rot disease incidence scores and lesion length were recorded. The pathogen was re-isolated from infected part to confirm the pathogenicity.

Attached Tiller Assay and Standard Grain Inoculation Techniques

The experiment was carried out in polyhouse in Dept. of Genetics and Plant Breeding, University of Agricultural Sciences, Bangalore during *kharif*, 2013. Seven days old culture of *S. oryzae* (GKVK isolate, Saro24, gene bank ID: KT291728) cultured on potato dextrose agar (HiMedia ID: M-096) used in the experiment. Mycelial mat on potato dextrose agar medium was cut into approximately equal size using sterile blade and used for inoculation purpose. Tillers at booting stage grown in pots inside the polyhouse was with mycelial disc, covered with sterile moistened absorbent cotton and wrapped with paraffin film in attached tiller assay methods. Whereas in standard grain inoculation method, the mycelial disc was inserted into leaf sheath during booting stage. The disease development was qualitative scored as infected and immune on 15^{th} days after inoculation. The pathogen was re-isolated from infected sheath to confirm the pathogenicity.

Detached Leaf and Leaf Sheath Assay

Leaf and sheath of BPT-5204 and HP-14 were cut into 7 cm long pieces, surface sterilized with 0.05 per cent mercuric chloride, placed sterile petriplates containing moist Whatman filter papers. Mycelial disc was placed at one end of leaf or sheath. The disease development was qualitative scored as infected and immune on 72 hours after inoculation.

The pathogen was re-isolated from infected sheath to confirm the pathogenicity

RESULTS AND DISCUSSIONS

Sheaths rot disease of rice caused by the fungal pathogen *S. oryzae* [(Sawada) W. Gams & D. Hawksw] becoming a serious threat to rice production worldwide. It is advantageous and important to employ appropriate disease screening methods to identify resistant sources in germplasm, land races, breeding lines and wild genetic resources. Therefore, standardization of screening protocols for sheath rot disease was undertaken and results were discussed below

Seed Inoculation

Significant differences were observed between two factors *viz.* seed inoculation and among varieties. Plant height of BPT 5204, MAS 26 and MAS 946-1 recorded 8.4 cm, 4.62 cm and 8.8 cm respectively in the control and 3.0 cm, 2.7 cm and 2.24 cm in the inoculated seedlings (Table 1 and Plate 2). Besides, inoculated seedlings are more yellowing and whitish tips noticed as compared to control. Therefore, seed inoculation of seeds of breeding lines with overnight soaking of seeds in conidial suspension was found very effective in screening of germplasm for sheath rot disease resistance in rice. This method suitable for large scale screening of breeding materials and germplasm, mapping population. This method is not suitable for screening of segregating material due reduced germination percentage and reduced vigour.

Table 1: Standardization of Seed Inoculation Technique for Screening of Breeding Lines for Response to Sheath Rot Disease in Rice

Factor	BPT-5204	MAS-26	MAS-946-1	Mean
Seed inoculated	3.9	2.7	2.24	2.947
control	8.4	4.6	8.8	7.267
Mean	**6.15**	**3.65**	**5.52**	
	S.Em.±	CD (P=0.05)		
Inoculation	0.209	0.613		
Varieties	0.256	0.751		
Inoculation × varieties	0.362	1.062		

Detached Tiller Assay

Experimental results of three factorial CRD was summarized in table 2, figure 1 and plate 3. There were significant differences between inoculation techniques adopted in the experiments and the control. Mean sheath rot lesion length observed under artificial inoculation was 4.65 cm as compared to control (0.00 cm). Similarly,

Significant varietal differences recorded for sheath rot lesion length (Plate-10), mean lesion length expressed by BPT-5204 was 3.25 cm as compared to HP-14 (1.4 cm). Comparison of lesion length between injured sheath *vs* control found non-significant with lesion length of 2.4 and 2.25 cm, respectively. Therefore, screening of germplasm or breeding material under detached tiller assay with or without injuring the flag leaf sheath did not affect the disease development in resistant and susceptible varieties. This method suitable for large scale screening of breeding material and germplasm, mapping population including segregating breeding population. It requires only one tiller per plant and rest of tillers used for field screening and seed purpose.

Similarly for length of panicle discolouration, significant differences were observed for only inoculation techniques with mean length of panicle discolouration of 6.55 cm under inoculation as compared to 3.4 cm under control, whereas there were no significant differences among varieties (BPT-5204 and HP-14) and injured sheath. During booting stage, panicles were hidden in flag leaf sheath and very sensitive to fungal infection. Panicle discolouration considered as

syndrome caused by dozens of fungi and thousands of pathogenic bacteria (Cottyn *et al*., 1996).

Attached Tiller Assay

The susceptible varieties BPT-5204 and resistant varieties were screened for attached tiller method (Plate-4) and qualitatively scored for sheath rot infection. BPT-5204 took infection with enlarged lesion within 15 days after inoculation whereas resistant parent HP-14 took no infection and shows on brown pustules considered as highly resistant to sheath rot disease. However, this methods was not suitable for screening of large number of germplasm as it require pot culture experiments.

Standardization of disease screening through detached tiller assay and attached tiller assay were first of its kind. Samiyappan *et al.,* (2003) standardized detached leaf sheath assay and electrolyte leakage assay for sheath rot disease screening. Shakthivel and Gnanamanickam

Plate 2: Response Breeding Lines for Seedling Vigour Under Seed Inoculation Technique (with Condial Suspesion 10^5 Spores/ml) in Rice

A. Without Injuring Flag Leaf Sheath B. Injuring of Flag Leaf Sheath

Plate 3: Response of BPT-5204 and HP-14 for Sheath Rot Disease Under Detached Tiller Assay in Rice

Table 2: Standardization of Detached Tiller Assay for Screening of Breeding Lines for Response to Sheath Rot Disease in Rice

Treatment		Sheath Rot Lesion Length (cm)				Panicle Discolouration (cm)			
		Variety		Mean		Variety		Mean	
		BPT-5204	HP-14	Inoculated	Injured sheath	BPT-5204	HP-14	Inoculated	Injured sheath
Inoculated	Injured sheath	6	3.6	**4.65**	**2.4**	6	6.6	**6.55**	**5.0**
	Control	7	2		**2.25**	7	6.6		**3.4**
Control	Injured sheath	0	0	**0.00**		3.8	3.6	**1.85**	
	Control	0	0			0.0	0.0		
	Mean	**3.25**	**1.4**			**4.2**	**4.2**		
		S.Em.±		CD (P=0.05)		S.Em. ±		CD (P=0.05)	
Inoculation		0.3288		0.9472		0.6078		1.7508	

Table 2: Contd.,				
Injured	0.3288	NS	0.6078	NS
Varieties	0.3288	0.9472	0.6078	NS
Inoculation × Injury	0.4650	NS	0.8595	2.4759
Inoculation × varieties	0.4650	1.3396	0.8595	NS
Injury × varieties	0.4650	NS	0.8595	NS
Inoculation × Injured × varieties	0.6576	NS	1.2155	NS

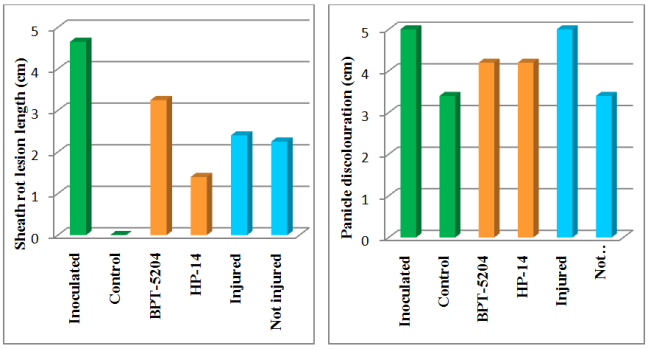


Figure 1: Response of Parental Varieties (BPT-5204 and HP-14) for Sheath Rot Disease Under Detached Tiller Assay

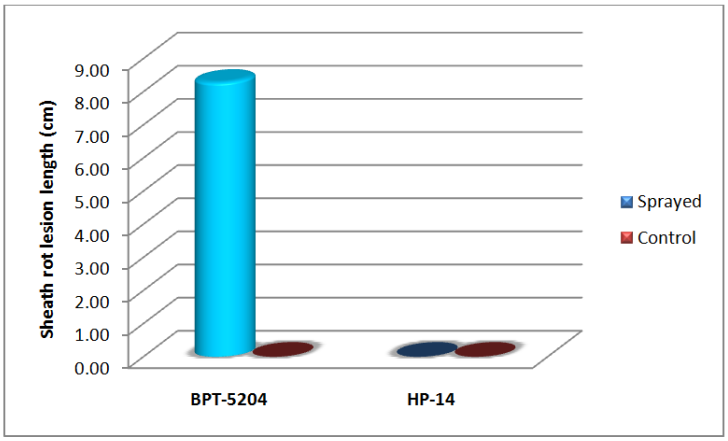
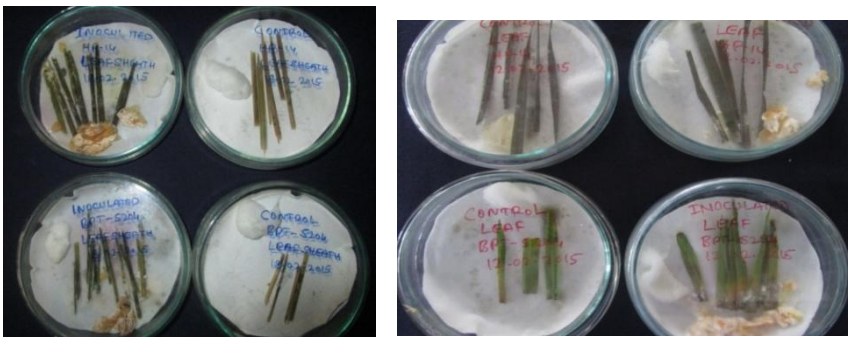


Figure 2: Response of Parental Varieties for Sheath Rot Disease under Artificial foliar Inoculation Conidial Suspension (10^5 conidia/ml)

Plate 4: Response of BPT-5204 for Sheath Rot Disease under Attached Tiller Assay in Rice

Plate 5: Response of Parents (BPT-5204 and HP-14) to Sheath Rot Disease under Artificial Disease Screening (Foliar Inoculatiuon with Conidial Suspension of 10^5 Conidia/Ml) During Booting Stage in Pot Culture experiments

Plate 6: Response of BPT-5204 to Sheath Rot Disease under Standard Grain Inoculation Technique During Booting Stage in Pot Culture experiments



A. Detached Leaf Sheath Technique B. Detached Leaf Technique

Plate 7: Response of BPT-5204 and HP-14 for Response to Sheath Rot Disease Under Detached Leaf and Leaf Sheath Assy Experiment

(1986) reported standard grain inoculation method of screening for sheath rot disease. Nandakumar *et al* (2007) reported modified standard seed kernel method by replacing insertion of seed kernals with mycelia mat into flag leaf sheath, covered with absorbent cotton and wrapped with paraffin film.

Foliar Inoculation and Cotton Swabbing with Conidial Suspension

Foliar inoculation of conidial suspension (10^5 conidia/ml) during booting stage was undertaken in pot culture experiment for BPT-5204 and HP-14. The experimental results were showed that HP-14 was highly resistant and BPT-5204 was high susceptible (Figure 2 and Plate-5). Mean lesion length of BPT-5204 was 8.5 cm as compared to HP-14 (0.0 cm). Similar results of foliar inoculation with conidia suspension in 25 *per cent* of peptone broth and one *per cent* of silicon carbide resulted higher level of sheath rot disease incidence was reported by Estrada and Crill (1980) and Gill (1990).

Standard Grain Inoculation Technique

Mycelial disc was inserted into flag leaf sheath during booting stage and observations were recorded for disease development for sheath rot disease development after 15 days (Plate 6). The enlarged sheath rot lesion was observed in the inoculated sheath in all the inoculated sheath. This method of screening was in confirmation with findings of Shakthivel and Gnanamanickam (1986). However, this methods was not suitable for screening of large number of germplasm as it require pot culture experiments.

Detached Sheath and Leaf Assay

The sheath and leaf of BPT-5204 and HP-14 were inoculated by detached tiller assay and detached sheath assay (Plate-7) and scored qualitatively for disease infection. BPT-5204 took infection with in 72 hours of inoculation and found highly susceptible to sheath rot disease resistance whereas, HP-14 took no infection and considered as highly resistant to sheath rot disease. The detached sheath assay method of screening was in agreement with Samiyappan *et al.,* (2003) and detached leaf assay is first kind of report. These two methods were suitable for screening of large number of germplasm and breeding lines in laboratory condition. However, surface sterilization of sheath and leaf is tedious and contamination with other fungus during incubation time hinders for large scale screening of germplasm and breeding material.

CONCLUSIONS

Sheath rot disease of rice has emerged as one of the major disease causing considerable qualitative and quantitative yield loss. Employing of appropriate disease screening methods to identify resistant sources is very important in organizing breeding programmes. The seed inoculation techniques, detached tiller assay and folia inoculation with cotton swab method of inoculation were found suitable for large screening of germplasm, breeding lines and phenotyping of mapping population. Attached tiller assay and standard grain inoculation were highly suitable for pathogenicity test related studies. Detached leaf and leaf sheath assay suitable screening of fewer numbers of germplasm and breeding materials under controlled laboratory conditions.

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